

AMENDMENTS

In the Claims:

1. (Previously Presented) A method for detecting and counting individual intracellularly labeled microorganisms in a sample, wherein background fluorescence is avoided, comprising the steps of:

- a) selectively enriching the microorganism sought in the sample,
- b) inducing or activating at least one enzymatic activity of the microorganism,
- c) immunomagnetically concentrating the microorganism,
- d) fluorescently labeling the microorganism by adding to the sample containing the microorganisms at least one substrate comprising one part specific to the enzymatic activity to be indicated and one fluorogenic label, wherein the transformation of the substrate takes place inside the microorganism and wherein the fluorescent product resulting from the fluorogenic label is retained in the microorganism thereby avoiding the production of background fluorescence and permitting the counting of each individual microorganism, and
- e) detecting and counting the each fluorescence labeled ~~microorganisms~~ microorganism by a technique selected from the group consisting of: flow cytometry, filtration cytometry and fluorescence microscopy.

2. (Currently Amended) A method according to claim 1, wherein the enrichment step is carried out in a composition comprising:

sodium pyruvate at a concentration selected from the group consisting of between 1 and 20 g/L, between 1 and 10 g/L, and between 4 to 6 g/L,

sodium thiosulfate at a concentration selected from the group consisting of between 0.5 and 5 g/L, between 0.5 and 3 g/L, and 2 g/L,

catalase at a concentration selected from the group consisting of between 500 and 20,000 IU/L, between 2,000 and 8,000 IU/L, and ~~about~~ 5,000 IU/L.

3. (Previously Presented) A method according to claim 2, wherein said composition comprises in addition at least one antibiotic.
4. (Previously Presented) A method according to claim 1, wherein step b) is an induction step for at least one enzymatic activity specific to the microorganism sought, comprising adding to the microorganism's enrichment medium at least one non-fluorescent substrate specific to said enzyme or enzymes.
5. (Previously Presented) A method according to claim 4, wherein steps a) and b) are carried out simultaneously.
6. (Previously Presented) A method according to claim 4 or 5, wherein in step c), immunomagnetically concentrating the microorganism takes place before inducing or activating at least one enzymatic activity of the microorganism in step b), or wherein in step c), immunomagnetically concentrating the microorganism takes place after fluorescently labeling the microorganism in step d).
7. (Previously Presented) A method according to claim 1, wherein in the case where the microorganism sought is a Gram-positive bacteria, step b) comprises in addition to an induction step, further comprises adding to the microorganism's enrichment, medium yeast extract at a concentration selected from the group consisting of between 5 and 50 g/L, between 10 and 20 g/L, and 10 g/L.
8. (Previously Presented) A method according to claim 1, wherein the immunomagnetic concentration step comprises the steps of:
 - a) placing the microorganism sought, present in the sample, in contact with an antibody directed against an antigen specific to the microorganism, the antibody being conjugated with a magnetic bead,
 - b) separating the bead-antibody-microorganism complexes from the sample, and
 - c) separating the microorganism from the rest of the complex.

9. (Original) A method according to claim 8, wherein the antibody conjugated with a magnetic bead is directed against an antibody that is itself directed against an antigen specific to the microorganism sought.
10. (Previously Presented) A method according to claim 8 or 9, wherein the magnetic beads have a diameter that is between 1 and 20 μm , or between 2 and 8 μm .
11. (Canceled)
12. (Currently Amended) A method according to claim ~~11~~ 1, wherein the label is a fluorogenic label excited at 488 nm selected from the group consisting of the xanthenes, acridines, phycobiliproteins, cyanine, and esculin.
13. (Currently Amended) A method according to claim ~~11~~ 4, wherein the substrate part specific to the enzymatic activity to be revealed is selected from the group consisting of a fatty acid, a monosaccharide, a phosphate, and a sulfate.
14. (Canceled)
15. (Previously Presented) A method according to claim 1, wherein steps a), b), c), d), and e) are preceded by a filtration step for the sample to be analyzed.
16. (Previously Presented) A method according to claim 15, wherein the filtration is carried out by means of a filter whose porosity is a size selected from the group consisting of between 20 and 150 microns, between 30 and 100 microns, and 63 microns.
17. (Previously Presented) A method according to claim 15, wherein the filtration is carried out on a membrane presenting a porosity selected from the group consisting of between 0.2 and 10 μm , between 0.2 and 5 μm , and between 0.2 and 0.5 μm .
18. (Withdrawn) A selective enrichment medium for a microorganism sought in a sample comprising:
a nutrient composition making the multiplication of said organism possible, and

a selective revivification composition for said microorganism, wherein it comprises:

sodium pyruvate at a concentration selected from the group consisting of between 1 and 20 g/L, between 1 and 10 g/L, and between 4 to 6 g/L,

sodium thiosulfate at a concentration selected from the group consisting of between 0.5 and 5 g/L, between 0.5 and 3 g/L, and approximately 2 g/L,

catalase at a concentration selected from the group consisting of between 500 and 20,000 μ /L, between 2,000 and 8,000 g/L, and approximately 5,000 μ /L.

19. (Withdrawn) An enrichment medium according to claim 18, which further comprises at least one antimicrobial agent.

20. (Withdrawn) A kit for detecting and counting microorganisms comprising:

a) an enrichment medium according to claim 18 in a liquid or dehydrated form, a plastic bag lined with a full-surface filter presenting a porosity of approximately 63 μ m,

b) magnetic beads conjugated to an antibody specific for an antigen on said microorganism,

c) one or several substrates in a lyophilized form,

d) solvents,

wherein said substrates of part (c) comprises a part specific to enzymatic activity to be revealed and a label.

21. (Currently amended) A method for detecting and counting individual intracellularly labeled microorganisms in a sample, wherein background fluorescence is avoided, comprising the steps of:

a) selectively enriching the microorganism sought in the sample,

b) inducing or activating at least one enzymatic activity of the microorganism,

c) immunomagnetically concentrating the microorganism,

d) fluorescently labeling the microorganism by adding to the sample containing microorganisms at least one substrate comprising one part specific to the enzymatic activity to be indicated and one label part fluorogenic label, wherein the transformation of the substrate takes place inside the microorganism and wherein the fluorescent product resulting from the fluorogenic label is retained in the microorganism, thereby avoiding the production of background fluorescence and permitting the counting of each individual microorganism and

e) detecting and counting the each fluorescence labeled microorganisms microorganism;
wherein the microorganisms are enriched in a composition comprising sodium pyruvate, sodium thiosulfate, and catalase.

22. (Previously Presented) The method of claim 21, wherein the microorganisms are enriched in a composition comprising:

sodium pyruvate at a concentration selected from the group consisting of between 1 and 20 g/L, between 1 and 10 g/L, and between 4 to 6 g/L,

sodium thiosulfate at a concentration selected from the group consisting of between 0.5 and 5 g/L, between 0.5 and 3 g/L, and 2 g/L, and

catalase at a concentration selected from the group consisting of between 500 and 20,000 IU/L, between 2,000 and 8,000 IU/L, and 5,000 IU/L.